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Self-assembled curcumin-soluble soybean polysaccharide nanoparticles: Physicochemical properties and *in vitro* anti-proliferation activity against cancer cells

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ABSTRACT

Nanoencapsulation of lipophilic bioactive compounds in food biopolymers is important to functional beverages, but protein-based nanocapsules are unstable around the isoelectric point of protein. The objectives of this work were to study physicochemical properties of self-assembled curcumin-soluble soybean polysaccharide (SSPS) nanoparticles and evaluate the activities against proliferation of human colon HCT116 and mammary adenocarcinoma MCF-7 cancer cells before and after simulated digestions. Capsules with a hydrodynamic diameter of 200–300 nm and an encapsulation efficiency of ~90% were self-assembled after increasing curcumin-SSPS mixture to pH 12.0 and lowering pH to 7.0. The capsule dispersions were stable at pH 2.0–7.0 and after heating at 95 °C for 1 min. No significant difference was observed for the viability of HCT 116 and MCF-7 cells challenged with 0.4, 4.0, and 40 μg/ml nanoencapsulated curcumin before and after simulated gastric and intestinal digestions. These findings may be significant to help develop functional beverages for disease prevention.

1. Introduction

Lipophilic phytochemicals with anti-oxidant, anti-cancer and anti-inflammatory activities are significant in the prevention of chronic diseases (Scalbert et al., 2011; Ting, Li, Ho, & Huang, 2013). To develop functional beverages, delivery systems are needed to encapsulate these lipophilic compounds to achieve dispersion stability during processing and shelf-life storage, prevent their degradation during storage and post-ingestion, improve their absorption, and deliver them to the target sites (Pan, Zhong, & Baek, 2013). To enable dispersion stability and sometimes clarity of beverages, nanoscale capsules can be fabricated with food grade ingredients in colloidal structures of biopolymer nanocapsules, nanoemulsions and microemulsions (Pan & Zhong, 2016).

Food biopolymer nanocapsules have advantages such as label-friendliness of "natural" and "fat-free" that may not be the case for nanoemulsions and microemulsions. Food proteins are frequently studied to prepare delivery systems because they are generally recognized as safe, abundant, sustainable and inexpensive (Chen, Remondetto, & Subirade, 2006). Some desirable characteristics of protein-based delivery systems include the improvement in shelf life stability of labile nutraceuticals and the enhancement in the bioavailability and bioactivity (Elzoghby, Samy, & Elgindy, 2012). However, protein-based delivery systems can aggregate at a pH nearby the isoelectric point of proteins and after thermal processing. Their applicability for intestinal delivery is also questionable due to the digestion by proteases.

Anionic polysaccharides can be stable at a wide range of pH and many are dietary fibres, stable against hydrolysis by enzymes in gastric and intestinal juices. Gum arabic and soluble soybean polysaccharides (SSPS) are two possible polysaccharides for functional beverage applications because of their low-viscosity and their ability to bind lipophilic compounds, in addition to their pH stability and their dietary fibre feature (Gomes et al., 2010; Nakamura, Yoshida, Maeda, & Corredig, 2006a; Nakamura, Yoshida, Maeda, & Corredig, 2006b). Gum arabic is a branched polysaccharide with a small proportion of polypeptide chain enabling its amphiphilic properties and its common application for emulsification (Jayme, Dunstan, & Gee, 1999). SSPS is prepared from soybean cotyledons and contains glycoproteins enabling its emulsification activity (Maeda & Nakamura, 2009; Wu, Lin, & Zhong, 2014). Similar to pectin, SSPS can adsorb on protein nanoparticles to provide steric repulsion to prevent aggregation at an acidity near a protein's

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isoelectric point (Maeda & Nakamura, 2009; Nakamura, Furuta, Kato, Maeda, & Nagamatsu, 2003; Nakamura et al., 2006b; Pan, Chen, Davidson, & Zhong, 2014; Zhang & Zhong, 2013). However, there are few studies reporting nanocapsules of phytochemicals fabricated with these two polysaccharides with features for functional beverage applications.

The first objective of the present study was therefore to prepare curcumin-SSPS nanocapsules and characterize physicochemical properties of the nanodispersions. Curcumin is a commonly studied lipophilic phytochemical with a water solubility of only about 11 ng/ml (Kaminaga et al., 2003) but with excellent cytotoxic and anti-carcinogenic activities against various cell lines (Babu, Shylesh, & Padikkala, 2002; Ruby, Kuttan, Dinesh Babu, Rajasekharan, & Kuttan, 1995; Soudamini & Kuttan, 1988). Instead of using the commonly studied anti-solvent precipitation method that requires the removal of alcohol (Chen, Ou, Chen, & Tang, 2017), a pH-cycle method from our previous study (Pan, Luo, Gan, Baek, & Zhong, 2014) was used to prepare self-assembled curcumin-SSPS nanocapsules. The principle is based on the pH-dependent solubility of curcumin, which is insoluble at neutral pH but is soluble at alkaline pH with its three hydroxyl groups being deprotonated. Therefore, sequential steps of increasing pH to 12.0 to dissolve curcumin and acidification to neutral pH enabled the *in situ* encapsulation of precipitated curcumin by sodium caseinate in nanocapsules with a dimension of 20–40 nm based on transmission electron microscopy. Gum arabic was not chosen because of a low encapsulation efficiency in preliminary studies using the pH-cycle method. The second objective of this study was to assess the activity of curcumin nanoencapsualted in SSPS against the proliferation of human colon and breast cancer cells before and after simulated digestions.

2. Materials and methods

2.1. Chemicals

Curcumin with a reported purity of over 90% was procured from Sigma-Aldrich Corp. (St Louis, MO, USA). SSPS (SOYAFIBE-S Serie) was purchased from Fuji Oil Corp. (Osaka, Japan). Other chemicals were products obtained from either Sigma-Aldrich or Thermo Fisher Scientific (Pittsburgh, PA, USA).

2.2. Encapsulation protocol

The encapsulation protocol followed the previous work (Pan et al., 2014), with slight modifications. SSPS was hydrated at 2% w/w in deionized water overnight at room temperature (RT, 21 °C) and was adjusted to pH 12.0 using 4.0 M NaOH. Then curcumin crystals were mixed with the SSPS dispersion at an overall concentration of 0.4 mg/ml. After stirring on a magnetic stirring plate for 30 min, the mixture was adjusted to pH 7.0 using 2.0 M HCl, followed by centrifugation at 2920*g* (Sorvall RC-5B plus, Sorvall, Newtown, CT, USA) for 10 min at RT to remove big particulates. Lyophilized samples were additionally prepared (model 12 EL freeze drier, VirTis Company, Inc., Gardiner, NY, USA) for subsequent experiments utilizing powdered samples.

2.3. Determination of encapsulation efficiency (EE)

The above supernatant after neutralization and centrifugation was transferred to determine the amount of encapsulated curcumin based on absorbance at 419 nm (Abs_{419}) by referring to a standard curve established from standard curcumin solutions in chloroform (Pan et al., 2013). An appropriate amount of chloroform was mixed with the supernatant to fit the curcumin concentration range of the standard curve. After stirring overnight at RT, the bottom chloroform phase was quantified for Abs_{419} using a UV–Vis spectrophotometer (Evolution 201, Thermo Scientific, Waltham, MA, USA). The EE was determined based on the percentage of curcumin mass in the supernatant with respect to the total curcumin mass in an encapsulation experiment. The EE was determined from three independent replicates.

2.4. Differential scanning calorimetry (DSC)

A model Q2000 calorimeter (TA Instruments, New Castle, DE, USA) was used to characterize thermal properties of lyophilized powder with comparison to pristine curcumin crystals. In each aluminium pan, 10 mg of powder was contained and hermetically sealed. Samples were heated from 30 to 250 °C at a rate of 5 °C/min. Nitrogen was used as a transfer gas at a flow rate of 50 ml/min.

2.5. UV–Vis absorption spectra of curcumin before and after encapsulation

UV–Vis spectroscopy was used to compare structural properties of curcumin before and after encapsulation. The supernatant after encapsulating curcumin was prepared as above and extracted using chloroform. The pristine curcumin solution was prepared by dissolving curcumin crystals in chloroform directly to the same concentration as that extracted from the encapsulation sample. The absorption spectra from 350 to 500 nm were collected at RT, with chloroform being a blank (Evolution 201, Thermo Scientific, Waltham, MA, USA).

2.6. Dynamic light scattering (DLS)

Particle size distributions of SSPS at pH 7.0 before alkalization, at pH 12.0, and after being neutralized back to pH 7.0, as well as SSPS encapsulated with curcumin at pH 7.0, were recorded with a Malvern Nano ZS instrument (Malvern Instrument Ltd, Worcestershire, UK).

2.7. Atomic force microscopy (AFM)

SSPS and SSPS encapsulated with curcumin were diluted in deionized water to an overall solute concentration of 10 ppm. After dilution, 2.0 μl of each sample was spread evenly onto a freshly cleaved mica sheet mounted on a sample disk and was dried overnight in air at RT (Bruker Corp., Santa Barbara, CA, USA). A rectangular cantilever having an aluminium reflective coating on the backside (ScanAsyst, Bruker Corp.) equipped on a Multimode VIII microscope (Bruker AXS, Billerica, MA, USA) was operated in the tapping mode at a scanning speed of 1 Hz to collect topographical images at a preset scan area of 2.0 × 2.0 μm.

2.8. Heat stability of encapsulated curcumin at pH 2.0–7.0

Dispersions diluted 60 times with deionized water were adjusted to pH 2.0–7.0 using 1.0 M HCl, followed by heating at 95 °C in a water bath for 1 min. Abs_{419} and Hunter Lab color space (*L*, *a*, *b*) values before and after heating were recorded as the chemical stability of curcumin using the above UV–Vis spectrophotometer and a Hunter colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA), respectively. Absorbance at 500 nm (Abs_{500}) and mean particle size before and after heating were also measured as physical stability of the dispersions using the above UV–Vis and DLS instruments, respectively. Three independent replicates were evaluated.

2.9. Anti-proliferation activity of encapsulated curcumin before and after in vitro digestions

The anti-proliferation activity of curcumin encapsulated in SSPS before and after treatment in simulated gastric and intestinal digestive fluids was compared to free curcumin dissolved in dimethyl sulfoxide (DMSO). The simulated gastric and intestinal digestion conditions followed literature methods, with slight modification (Guri, Haratifar, & Corredig, 2014; Luo, Pan, & Zhong, 2015). Pepsin and pancreatin were dispersed separately at 8.0 mg/ml and 80.0 mg/ml in 10 mM